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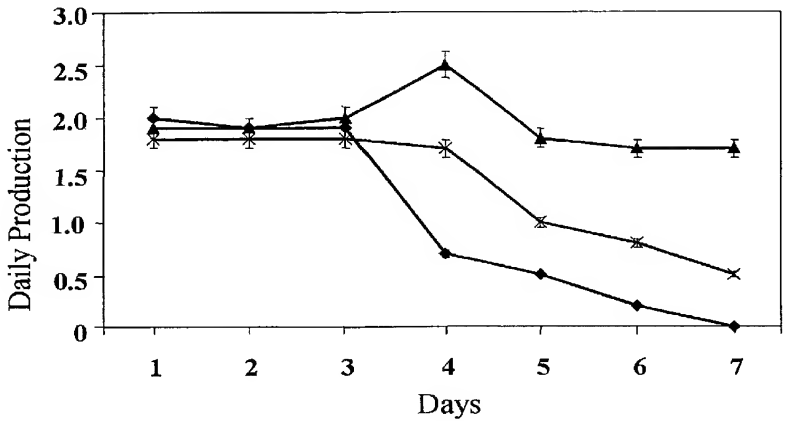
(54) Title: FEED SUITABLE FOR CULTURING ROTIFERS, LARVAL SHRIMP, AND MARINE FILTER FEEDERS



(57) Abstract: The invention provides an inexpensive and highly nutritious source of food for mass production of rotifers, shrimp, larvae, and other filter feeders, such as oysters, clams, mollusks, and mussels. The invention provides a feed comprising a protein source, e.g., yeast, a macroalgal product, and a microalgal product in combination to enhance the growth of zooplankter and thereby the ultimate consumer of said zooplankter, a targeted cultured species.

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FEED SUITABLE FOR CULTURING ROTIFERS, LARVAL SHRIMP, AND MARINE FILTER FEEDERS

PRIORITY CLAIM

[001] This application claims the priority of U.S. provisional application 60/426,022 entitled "Feed Suitable for Culturing Rotifers, Larval Shrimp, and Marine Filter Feeders," filed in the United States Patent and Trademark Office on November 14, 2002, the disclosure of which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

[002] The invention relates to feeds for aquatic organisms, such as rotifers, larval shrimp, and filter feeders. The feeds can be comprised of algae or yeast, or algal or yeast extracts, and can provide supplementation to the aquatic organisms, for example, with vitamins, *e.g.*, vitamin B12, fatty acids, amino acids, growth factors, antibacterial agents, and pigments.

BACKGROUND ART

[003] Over the last two decades, aquaculture has become one of the fastest growing economic ventures. Aquacultural production is expected to expand rapidly in order to meet future demands of a growing global population. Researchers believe, however, that there is little likelihood of expanding wild fish catches as the entire fishing industry is in decline due to over fishing. Additionally, unless technological breakthroughs are made, aquaculture expansion is not expected to be sufficient to satisfy the projected demand. The resulting demand for seafood supply, associated with a steady decline in wild fish stocks due to over fishing, habitat destruction, and pollution, drives commercial fish culturists and hatcheries to increase and intensify their production capacities. However, low survival and poor growth rates of marine fish larvae, as a result of insufficient supply of live feed organisms and reduced food quality, is hindering the required development of commercially important marine aquacultural fish species. On a commercial production scale, non-optimal growth and

survival means additional tank space, extended larval rearing periods, and associated increases in operational costs (*i.e.*, the difference between survival of a business and its failure).

[004] One of the drawbacks to the development and intensification of marine aquaculture is the fact that the majority of the commercially important marine fish species require live organisms as their first feed (larval feeding). Despite extensive efforts to develop artificial alternatives (Dabrowski and Culver 1991; Kanazawa 1992), hatcheries are still vitally relying on live feeds, mainly various species of microscopic algae, the rotifer *Brachionus plicatilis,* and the brine shrimp *Artemia salina* or *Artemia franciscana*. Of all live foods used in fish and crustacean hatcheries, brine shrimp constitute the main food item because of their practical convenience and availability. As of today, brine shrimp are harvested mainly from the wild at an annual production of over 2000 metric tons of dry cysts. Nonetheless, a large part of the *Artemia* cyst market is still supplied from one location, the Great Salt Lake. This situation makes the market extremely vulnerable to climatological and ecological changes in this lake, which has been illustrated by the unusually low cyst harvest in the last four years. As a result, prices jumped from a moderate range of 20-40 dollars per kg dry cysts up to 80-100 U.S. dollars per kg, deeply affecting the entire fish and shellfish culture industry. Another major drawback is that newly hatched brine shrimp nauplii cannot be ingested by early stage larvae of most marine fish species, and therefore, they must be offered with more appropriate planktonic substitutes.

[005] The larval period of many marine fish and invertebrates is spent in estuaries where rotifers are the most abundant and dominant zooplankton. As a result of this natural ecological association, which has evolved over millions of years, many fish larvae and invertebrates are adapted to capture and nutritionally utilize rotifers. The planktonic nature of rotifers, their tolerance to a wide range of environmental conditions, high reproduction rate (0.7-1.4 offspring/ female/day), the ability to produce resting eggs which can remain dormant for several years, smaller size (100-500 μm lorica length), and slow swimming speed makes them an ideal live prey candidate for the culture of marine larvae. The success of rotifers as live food

organisms for the culture of early larval stages of marine fish encouraged investigation into the improvement of their mass culture under controlled conditions. Indeed, twenty-five years after the first mass production trial, rotifers are produced worldwide in large quantities and have contributed to the successful hatchery production of more than 60 marine finfish species, several species of freshwater fish, and 18 species of crustaceans (Nagata and Whyte 1992). The largest potential for rotifer culture resides, however, is the possibility of rearing these animals at very high densities (2000-10,000 individuals/mL) using condensed microalgal feed and oxygen supplementation, as has been successfully practiced recently in Japan and Europe (Yoshimura et al. 1994). Even at high densities, rotifers still reproduce rapidly, and can thus contribute large quantities of live food in a relatively short time before the onset of a commercial larval rearing trial.

[006] Production of the expected number of marine fish larvae and crustaceans for future aquaculture needs and for natural stock enhancement programs cannot be accomplished when utilizing traditional culture methods of live algae. These methods require large tank volumes, ranging from 100-1000 m^3, (Kitajima 1983) in which rotifers are generally maintained at low densities of 100-300 individuals/ml and fed mainly on yeast-based low quality products supplemented with live microalgae. As a result, an additional 2-3 times more tank space may be needed for rotifer culture than all other purposes (Yoshimura et al. 1996). Consequently, up to 90% of the manpower and tank space in a conventional hatchery is used for live food production. Moreover, several hatcheries have recently reported a significant decrease in rotifer production, probably as a result of uncharacterized changes in their water quality and environmental conditions (Yoshimura et al. 1996). In fact, Watanabe (Watanabe et al. 1983a; Watanabe et al. 1983b) stated that without first establishing the intensive culture of rotifers and their feed, larval rearing of marine fish will not be expanded significantly. Thus, it has become widely accepted that larval production is virtually limited by live algae and rotifer availability (Hirata et al. 1983; Fukusho and Hirayama 1992).

[007] Methods for preparing a particulate material suitable for use as an aquaculture feed from microbial biomass are disclosed in U.S. Patents 6,103,225,

6,451,567, and 6,372,460 (Barclay 2000; Barclay 2002; Gladue and Behrens 2002). These methods describe a fermentation process for algal cells having a high content of DHA residues. The main goal of those disclosures was raising the DHA level of the *Artemia* or rotifers then subsequently raising the DHA level of the larvae. For those organisms that are filter feeders, the material may be supplied as small particles and thus provide a direct enrichment of DHA into the diet. DHA-enriched aquaculture feeds comprising microflora selected from *Thraustochytrium, Schizochytrium,* and *Crypthecodinium* sp., and mixtures thereof are useful for aquaculture and in particular, for feeding larval shrimp and mollusks (Barclay 2000; Gladue and Behrens 2002). Because of the small aggregate size of the algal cells, the microflora can be eaten by larval shrimp, brine shrimp, rotifers, and mollusks (Langdon et al., 1999). As an alternative approach, the present invention discloses the use of nutritionally improved yeast as aquaculture feeds. The improved nutritional status of yeast is obtained by selecting partially digested yeast or yeast having thin cell walls and including a mixture of certain microalgae that are used for aquaculture, such as DHA-producing microalgae and macroalgal extracts, thus formulating a complete and highly nutritious feed for aquaculture. These algal feeds, which are based on yeast and microflora grown in fermentation medium or photoautotrophically grown algae, are suitable for use as feeds for rotifer, brine shrimp, shrimp larvae, clams, mussels, and mollusks. As discussed above, microflora grown under such conditions typically have a cell size less than about 50 μm.

[008] With the ability to formulate larval feeds solely from components derived from heterotrophic bacteria, algae, and yeast, we can eliminate the need for both the hatchery production of live algae and the use of meals and oils derived from wild caught fish. These algal-derived feeds would be much cheaper than currently used feeds. Their use in commercial hatcheries would improve operating efficiency by lowering costs associated with growing a sufficient amount of algae, thereby allowing for increasing aquacultural production.

DISCLOSURE OF THE INVENTION

[009] The invention provides an aquaculture feed comprising yeast and microalgae, or components thereof. The mean particle size can range from about 5 μm to about 100 μm, and the feed components can be dry mixed and ground to a fine powder. The yeast can comprise from about 30 to about 95 percent of the feed. Suitable yeast include, but are not limited to *Saccharomyces* spp., *Saccharomyces cerevisiae*, *Phaffia* spp., *Phaffia rhodozyma*, *Pichia* spp., *Pichia pastoris*, *Kluyveromyces* spp., *Kluyveromyces aestuarii*, *Kluyveromyces marxianus*, and *Kluyveromyces yarrowii*. The yeast can also be brewer's yeast.

[010] The feed can comprise at least about 5 percent by weight from microalgae. Suitable microalgae include, but are not limited to, *Tetraselmis* sp., *Tetraselmis suecica*, *Myrmecia* sp., *Myrmecia bissecta*, *Lyngbya* sp., *Lyngbya majuscula*, *Cytospora* sp., *Scenedesmus* sp., *Scenedesmus obliquus*, *Scytonema* sp., *Scytonema hofmanni*, *Nostoc* sp., *Nostoc weissfloggia*, *Chaetoceros* sp., *Chaetoceros lauderi*, *Ecklonia* sp., *Ecklonia maxima*, *Dunaliella* sp., *Dunaliella salina*, *Dunaliella tertiolecta*, *Dunaliella bardiwal*, *Pseudoanabaena* sp., *Anabaena* sp., *Prorocentrum* sp., *Prorocentrum minimum*, *Polysiphonia* sp., *Polysiphonia denudata*, *Spirulina* sp., *Arthrospira* sp., *Spirulina platensis*, *Aphanotheae* sp., *Aphanotheae nidulans*, *Hydrodictyon* sp., *Hydrodictyon reticulatum*, *Navicula* sp., *Navicula delongei*, *Phaeodactylum* sp., *Phaeodactylum tricornutum*, *Pseudonitzschia* sp., *Nitzschia* sp., *Nitzschia navis-varingica*, *Chlorella* sp., *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Chlamydomonas* sp., *Chlamydomonas reinhardii*, *Prennioporia* sp., *Prennioporia medullaeparis*, *Pandorina* sp., *Pandorina morum*, *Isochrysis* spp., *Isochrysis galbana*, *Schizochytrium* sp., *Crypthecodinium* sp., *Crypthecodinium cohnii*, and *Thraustochytrium* sp. Suitable microalgae also include, but are not limited to, *Schizochytrium* sp., *Crypthecodinium* sp., *Crypthecodinium cohnii*, *Thraustochytrium* sp., and components thereof. The feed can also comprise a probiotic element. Suitable probiotic elements include, but are not limited to, *Lactobacillus* sp., *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Pseudoalteromonas* sp., and *Pseudoalteromonas undina*.

[011] In an embodiment, the aquaculture feed can comprise yeast, microalgae, and macroalgae. The mean particle size can range from about 5 μm to about 100 μm, and the feed components can be dry mixed and ground to a fine powder. The yeast can comprise from about 30 to about 95 percent of the feed. Suitable yeast include, but are not limited to, *Saccharomyces* spp., *Saccharomyces cerevisiae*, *Phaffia* spp., *Phaffia rhodozyma*, *Pichia* spp., *Pichia pastoris*, *Kluyveromyces* spp., *Kluyveromyces aestuarii*, *Kluyveromyces marxianus*, and *Kluyveromyces yarrowii*. The yeast can be partially digested.

[012] The feed can comprise at least about 5 percent by weight from macroalgae. Suitable macroalgae include, but are not limited to, *Tetraselmis* sp., *Tetraselmis suecica*, *Myrmecia* sp., *Myrmecia bissecta*, *Lyngbya* sp., *Lyngbya majuscula*, *Cytospora* sp., *Scenedesmus* sp., *Scenedesmus obliquus*, *Scytonema* sp., *Scytonema hofmanni*, *Nostoc* sp., *Nostoc weissfloggia*, *Chaetoceros* sp., *Chaetoceros lauderi*, *Ecklonia* sp., *Ecklonia maxima*, *Dunaliella* sp., *Dunaliella salina*, *Dunaliella tertiolecta*, *Dunaliella bardiwal*, *Pseudoanabaena* sp., *Anabaena* sp., *Prorocentrum* sp., *Prorocentrum minimum*, *Polysiphonia* sp., *Polysiphonia denudata*, *Spirulina* sp., *Arthrospira* sp., *Spirulina platensis*, *Aphanotheae* sp., *Aphanotheae nidulans*, *Hydrodictyon* sp., *Hydrodictyon reticulatum*, *Navicula* sp., *Navicula delongei*, *Phaeodactylum* sp., *Phaeodactylum tricornutum*, *Pseudonitzschia* sp., *Nitzschia* sp., *Nitzschia navis-varingica*, *Chlorella* sp., *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Chlamydomonas* sp., *Chlamydomonas reinhardii*, *Prennioporia* sp., *Prennioporia medullaeparis*, *Pandorina* sp., *Pandorina morum*, *Isochrysis* spp., *Isochrysis galbana*, *Schizochytrium* sp., *Crypthecodinium* sp., *Crypthecodinium cohnii*, and *Thraustochytrium* sp. The aquaculture feed can comprise at least about 0.5 percent by weight from macroalgae, which can be chosen from *Laminaria* spp., *Padina* sp., *Gracillaria* sp., and *Ulva* sp. The macroalgae can comprise a macroalgal extract, which can further comprise an ethanolic extraction product. The feed can also comprise a probiotic element. Suitable probiotic elements include, but are not limited to, *Lactobacillus* sp., *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Pseudoalteromonas* sp., and *Pseudoalteromonas undina*.

[013] The invention also provides a method comprising feeding a cultured species with the feed described above.

[014] The invention further provides a method comprising feeding a zooplankter species with a feed described above. Suitable zooplankter species include, but are not limited to, brine shrimp, rotifers, *Artemia* spp., *Artemia salina, Artemia franciscana*, *Brachionus* spp., *Brachionus plicatilis*, copepods, and cladocerans.

BRIEF DESCRIPTION OF THE DRAWING

[015] **Figure 1.** The daily harvest (30% of total culture) of rotifers fed 0.4 g D.W. Bakers yeast and 40 x 10^6 live *Nanochloropsis* sp. algae per million rotifers (▲), 1 g algal feed and 0.1% *L. rhamnosus* per million rotifers (**X**), and 1 g Culture Selco (INVE, Bassrode Belgium) (♦).

MODES FOR CARRYING OUT THE INVENTION

Summary

[016] The purpose of the present invention is to provide an inexpensive and highly nutritious source of food derived by bacterial, algal, and/or yeast fermentation for mass production of rotifers, shrimp, larvae, and other filter feeders, such as oysters, clams, mollusks, and mussels. Currently, a major impediment to marine fish aquaculture is the high energy and labor cost (up to 85% of total operation costs according to Bolton and Fulks (Bolton 1982; Fulks and Main 1992)), and practical difficulties associated with growing high-quality algae. Frequently, it is difficult for a hatchery to supply sufficient algae at all times of the year. Unicellular algae are generally photoautotrophic and, as such, require light for photosynthesis during which carbon dioxide is reduced to organic carbon and oxygen is released. Hatcheries are generally using electric lighting for indoor algal cultures or, if climate conditions permit, algae can be grown in outdoor tanks. However, cell densities and growth rates are limited by light penetration into the culture medium. Photoautotrophic culture methods are energetically inefficient, costly, and require hatcheries to employ a skilled person to maintain the algal culture system.

[017] It is an objective of the invention to provide rotifers, shrimp, larvae, and filter feeders with inexpensive and high quality dry feeds that are produced from phototrophically or heterotrophically grown bacteria, algae, and yeast.

[018] It is an objective of the invention to provide rotifers, shrimp, larvae, and filter feeders with highly digestible yeast in a dry form. This yeast may be partially digested baker's yeast with cellulase or brewers yeast having a thin and easily digested cell wall.

[019] It is an objective of the invention to provide rotifers, shrimp larvae, and filter feeders with phototrophic or heterotrophic production of certain fresh-water and marine species of algae. Examples of microalgae include, but are not limited to, freshwater *Chlorella* sp., marine *Chlorella (Nannochloropsis oculata), Tetraselmis* sp., *Chaetoceros* sp., *Crypthecodinium* sp., *Schizochytrium* sp., *Chlorella* spp. (such as, but not limited to, *Chlorella vulgaris k-22),* and *Isochrysis galbana* (T-iso clone). These algal species are widely used in aquaculture operations, because of their high nutritional value, readiness for artificial culture, and also as a food source for rotifers (Hernandez et al. 1986; Nagata and Whyte 1992; Verginelli et al. 1994; Lie et al. 1997; Planas and Cunha 1999). *Crypthecodinium cohnii,* and S*chizochytrium* sp. have exceptionally high levels of docosahexaenoic acid (DHA, 22:6n-3), which is an essential fatty acid supplement in a marine fish larval diet (reviewed by Kanazawa (Kanazawa 1993) and Watanabe (Watanabe 1993)).

[020] It is an objective of the invention to provide rotifers and shrimp larvae and filter feeders with Vitamin B_{12} supplemented waste stream co-products of algae (up to 400 μg/100 g dry matter). More specifically, these feeds can be supplemented with additional algae-derived products containing phospholipids rich in essential fatty acids such as docosahexaenoic (DHA, 22:6n-3) and arachidonic (ARA, 20:4n-6) acids.

[021] It is an objective of the invention to provide rotifers, shrimp larvae, and other filter feeders with a dry form of macroalgal meal or extracts derived by such methods as, but not limited to, ethanolic or hot water extraction of macroalgal biomass. These extracts can contain additional essential growth factors, antibacterial agents, bacteriostatic compounds, pigments, and essential amino acids. Examples of

macroalgae include, but are not limited to, *Laminaria* sp., *Padina* sp., and *Gracillaria* sp.

Definitions

[022] In describing the present invention, the following terminology is used in accordance with the definitions set out below.

[023] The term "zooplankton" (plural) or "zooplankter" (singular) as used herein, refer to members of three major groups of invertebrate animals: the rotifers, copepods, and cladocerans. All three of these major groups have species adapted to pelagic (open water), littoral (vegetated), and benthic (bottom) environments, and are widespread in both freshwater and marine environments.

[024] The term "rotifer," as used herein, shall mean any of a class of minute, usually microscopic, but many-celled aquatic invertebrate animals having the anterior end modified into a retractile disk bearing circles of strong cilia that often give the appearance of rapidly revolving wheels. Rotifers are used as a source of live feed for aquatic animals during their early larval stage.

[025] The term "brine shrimp," as used herein, shall mean any strain of *Artemia*, belonging to the phylum Arthropoda in the class Crustacea. These tiny crustaceans can be found in salt lakes and brine ponds throughout the world. *Artemia* brine shrimp are used as a source of live feed for aquatic larvae during their early and late larval stages.

[026] The term "aquaculture feed," as used herein, shall mean any dry feed in a form of fine powder that is used to culture and grow zooplanktonic animals and aquatic larvae.

[027] The term "algal feed," as used herein, shall mean any dry feed form, which includes any portion of algal component.

[028] The term "enrichment feed," as used herein, shall mean any type of feed either dry or moist, that is used for short duration feeding of aquatic zooplankton, prior to their harvest, in order to deliver essential nutrients for aquatic larvae when fed to these zooplankton.

[029] The term "filter feeder" is any species of invertebrate animal that obtains its food from aquatic media by removal of small particles or organisms by any mechanism.

[030] The term "macroalgae" refers to algae that in at least one life stage form large structures that are easily discernable with the naked eye. Usually these organisms have secondary vascularization and organs. Examples of different groups containing macroalgae follow but are not limited to the chlorophyta, rhodophyta, and phaeophyta.

[031] The term "microalgae" refers to prokaryotic and eukaryotic algae that are classed in many different species. Normally the prokaryotic algae are referred to as cyanobacteria or bluegreen algae. The eukaryotic microalgae come from many different genera, some of which overlap with the macroalgae and are differentiated from these by their size and a lack of defined organs (although they do have specialized cell types). Examples of different groups containing microalgae follow but are not limited to the chlorophyta, rhodophyta, phaeophyta, dinophyta, euglenophyta, cyanophyta, prochlorophyta, and cryptophyta.

[032] The term "brewers yeast" is defined as any yeast preparation derived from yeast commonly used for production of beer, wine, or other fermented products. Such yeast can comprise, but are not limited to, *Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Saccharomyces diastaticus, Saccharomyces uvarum, Saccharomyces bayanus,* and *Kluyveromyces* sp.

Embodiments of the Invention

Production of Rotifers and Brine Shrimp

[033] The rotifer and brine shrimp culture system includes fiberglass tanks at a working volume of 20-2000 L each. This system is capable of producing up to 5 x 10^9 rotifers/day/tank at a density range of 200-10,000 individuals/mL or up to 10 kg brine shrimp wet biomass/day/tank. The temperature is regulated in each tank by a thermostat heater at 28-32°C, depending on the rotifer strain. A pH controller continuously monitors the pH in each culturing tank and 10% hydrochloric acid (HCl) is added automatically by means of a Petri pump to control the pH to between 6.5-7.0. Alternatively, a buffer solution containing sodium hydroxymethane sulfonate is added

to keep the ammonia in a non-toxic form. Slightly acidic pH of the culture medium is maintained, especially in high-density culture systems, since rotifer growth and fecundity rates are sensitive to high concentrations of toxic ionized ammonia (e.g., concentrations over 7.8 mg/L, as reported by Yu and Hirayama, 1986).

[034] Food is delivered to each culture tank manually or continuously by means of a Petri dosage pump. The daily amount of algal feeds and other supplements may be maintained in a chilled (0-4°C) suspension chamber, equipped with a slow mixing device. Rotifers are harvested continuously in a separate harvesting tank by daily exchanging of up to one third of the culture volume and can then be further enriched with essential nutrients before being delivered to fish larvae.

[035] With the increase in rotifer or brine shrimp density and subsequent increase in both feeding rations and organic load, a shortage of oxygen is expected. Therefore, pure oxygen, provided from oxygen cylinders or an oxygen generator, is diffused into each tank through air stones to maintain a minimal oxygen level at 3 ppm. Accumulation of particulate organic matter and contaminating protozoans is anticipated at high culture densities, which may affect production and clog plankton nets at harvesting. Therefore, the rotifer or brine shrimp medium is continuously circulated through nylon mat filters (1 cm thick); such a simple filtration system effectively removes solid substances with only minimal losses and stabilizes the culture performance (Yoshimura *et al.*, 1996; Yoshimura *et al.*, 1995).

Formulation of Algal Feeds

[036] Brewers yeast or partially hydrolyzed baker's yeast (where at least the external layer of the cell wall is digested, either enzymatically or chemically) provide brine shrimp and rotifers with highly digestible nutrients. The yeast content in the algal feed formulation ranges from 30% to 95% and, more preferably from 70% to 90% of the feed. The present invention provides yeast that are accessible to the digestive enzymes of their predators, but their wall remains sufficiently intact so that the cell contents do not escape into the water prior to consumption.

[037] Dried baker's yeast is prepared using enzymatic or chemical agents, such as, but not limited to, cellulase or mannase. Suitable enzymes and their

conditions of use (such as pH, concentration, temperature, and duration of the incubation) are known to those skilled in the art (Lavens et al. 1992).

[038] Long chain polyunsaturated fatty acid-rich microalgae are provided as a source of essential fatty acids, especially DHA, ARA and EPA (Barclay and Zeller 1996; Curé et al. 1996; Furuita et al. 1998; Abu-Rezq et al. 2002; Place and Harel 2002a; Place and Harel 2002b). The inclusion level of DHA-rich algae ranges from 0.5 % up to 50% of the feed, and more preferably from 5% to 20% of the feed. DHA (docosahexaenoic acid) has been identified as an important nutrient that contributes significantly to larval growth and survival (Curé et al. 1996). Larvae ultimately acquire these fatty acids from algae, either by directly feeding on algae with high levels of polyunsaturated fatty acids or by feeding on rotifers and brine shrimp that have been enriched with algae high in polyunsaturated fatty acids (Barclay and Zeller 1996). DHA-rich microalgae can be produced in fermentors utilizing sugar as a source of energy or photosynthetically. These algae are fortified with Vitamin B_{12}, a vitamin essential for growth and reproduction of rotifers, during the production process to contain at least 200 μg/100 g dry matter.

[039] Arachidonic acid (ARA) rich microalgae or fungi are provided as a source of essential fatty acids. The inclusion level of ARA-rich algae ranges from 0.5% to 10% of the feed, and more preferably from 2% to 8% of the feed. ARA has been identified as an important nutrient that contributes significantly to larval growth and survival as well as helping in osmotic regulation and stress resistance.

[040] Probiotic or prebiotic bacteria (*e.g.*, *Lactobacillus* sp., *Bacillus* sp., *Bifidobacterium* sp., and *Enterococcus* sp.) are added at a range from 0.01% to 5%. These bacteria function either to colonize the gut of the organism or deliver a beneficial activity or nutrient. Such probionts could relieve stress or reduce the pathogen load of the consuming animal.

[041] Macroalgal extracts are also included in the formulation at a range from about 0.1% to about 10%, and more preferably from about 0.5% to about 1%. These extracts provide the rotifers and brine shrimp with additional essential growth factors, antibacterial and bacteriostatic compounds, pigments, and essential amino acids.

[042] All algal species are enriched with vitamin B_{12} during production. This is because the rotifer *Brachionus plicatilis* requires vitamin B_{12} in its diet at a level of about 100-200 μg/100 g dry matter (Hirayama et al. 1989; Hirayama and Maruyama 1991; Maruyama and Hirayama 1993). The nutritional value of the algae is further enhanced by fortifying the algae with three fat-soluble vitamins (Vitamins A, D and E) and Vitamin C. All these vitamins showed supplementary effects and significantly increased the population growth of rotifers (Satuito and Hirayama 1986; Satuito and Hirayama 1991).

Examples

[043] Certain embodiments of the invention will now be described in more detail through the following Examples. The Examples are intended solely to aid in more fully describing selected embodiments of the invention and should not be considered to limit the scope of the invention in any way.

Example 1. Formulation of algal feed for rotifer culture

[044] Brewers dried yeast was obtained commercially from William Bio-products (Pekin, IL. USA). DHA-rich *Schizochytrium* sp. was obtained from Advanced BioNutrition Corp. (Columbia MD. USA). *Padina* sp. dry powder extract was obtained from ICP (Malta).

[045] Rotifer feed was formulated by mixing the ingredients in the proportions specified in Table 1. All ingredients were mixed on a dry weight basis and mill-ground to produce fine particles at sizes from about 5 to about 15 μm.

Table 1. Rotifer feed composition (g dry weight/100g)

Brewers yeast	90
Schizochytrium sp.	9
Padina sp.	1

[046] Rotifers were fed continually with a daily ration of 0.5 g dry matter/million rotifers. Rotifers were harvested continuously by daily exchanging a total of 30-50% of the culture volume. Temperature, oxygen level, total and free ammonia, and pH were monitored continuously as outlined above. Rotifers were used directly as live feed for larval stage fish, crustaceans, or other aquatic organisms.

Example 2. Formulation of algal feed for brine shrimp

[047] DHA-rich *Crypthecodinium* sp. and *Tetraselmis* sp., were produced under the trademark AquaGrow®. All other ingredients were obtained as specified in Example 1 and prepared according to Table 2.

Table 2. Brine shrimp feed composition (g dry weight/100 g)

Brewers dry yeast	50
Partially digested baker's yeast	40
Crypthecodinium sp.	5
Tetraselmis sp.	5

[048] Brine shrimp start to feed about 8 h post hatching (Instar-II nauplii stage), at an initial daily ration of 100% body weight. Daily ration is reduced every day by 10 % and culture tanks can be partially harvested when the required size is achieved. Complete harvesting can be done on day 10 post-hatch, when the brine shrimp reach maximum size or maximum biomass in the tank. Temperature, oxygen level, total and free ammonia, and pH are monitored continuously as outlined above.

Example 3. Formulation of algal feed for mussels

[049] Spray-dried *Spirulina* sp. was obtained from Cyanotech Corp. (Kailua-Kona, HI). All other ingredients were obtained as specified in Examples 1 and 2 and prepared according to Table 3. The dry meals were mixed and ground to fine particles of less than 50 μm.

Table 3. Mussel feed composition (g dry weight/100 g)

Brewers dry yeast	83
Crypthecodinium sp.	5
Schizochytrium sp.	5
Padina sp. extract	2
Spirulina sp.	5

[050] Juvenile mussels (*M. galloprovincialis*) are placed in 25 L plastic aquaria at a density of 20 individuals per aquarium. The culture system is supplied within a closed recirculating water system and equipped with a biofilter, 10 μm mesh filter, and UV-treatment. Temperature is maintained at 24-26°C. Algal meal is suspended in water and vigorously mixed in a blender and fed to the mussels twice daily at a total ration of 2 mg / L. The growth rate and survival of the animals is monitored on a weekly basis.

Example 4. Formulation of algal feed for shrimp larvae

[051] *Chaetoceros* sp. is grown on 50% Instant Ocean medium in photobioreactors using standard lighting and procedures known in the literature (D'Souza et al. 2002). *Laminaria* extract is obtained from Pacific Standard Distributors Inc (Menlo Park, CA). All ingredients were obtained as specified in Examples 1 and 2 and prepared according to Table 4. The dry meals were mixed and ground or milled to fine particles of less than 50 μm.

Table 4. Larval shrimp feed composition (g dry weight/100 g)

Partially digested baker's yeast	83
Crypthecodinium sp.	5
Laminaria sp. extract	2
Chaetoceros sp.	5

[052] Zooea stage shrimp larvae (*Penaeus vannamei* or *Litopenaeus vannamei*) are placed in 25 L plastic aquaria at a density of 50 individuals per aquarium. The culture system is supplied with a closed recirculating water system equipped with biofilter, 10 μm mesh filter and UV-treatment. The temperature is maintained between 24-26°C. Algal meal is suspended in water, vigorously mixed in a blender, and fed to the larvae twice daily at a total ration of 2 mg/L. The growth rate and survival of the animals are monitored on a weekly basis.

Example 5. Formulation of algal feed for rotifer culture

[053] Dried brewers yeast was obtained commercially from Williams Bio-products (Pekin, IL. USA). DHA-rich *Schizochytrium* sp. was obtained from

Advanced BioNutrition Corp. (Columbia MD. USA). Dry powdered *Padina* sp. extract was obtained from ICP Malta.

[054] Rotifer feed was formulated by mixing the 90 g of the brewers yeast, 9 g of the *Schizochytrium* biomass, and 1 g of the dried *Padina* extract (Table 5). All of the ingredients were mixed on a dry weight basis then mill-ground to produce fine particles at sizes between 5-15 μm.

Table 5. Rotifer feed composition (g dry weight/100g)

Brewers yeast	90
Schizochytrium sp.	9
Padina sp.	1

[055] Rotifers were fed continually with a daily ration of 0.5 g dry matter/million rotifers. Rotifers were harvested continuously by daily exchanging a total of 30-50% of the culture volume. Temperature, oxygen level, total and free ammonia, and pH were monitored continuously. Rotifers were used directly as live feed for larval stage fish, crustaceans, and other aquatic organisms. Performance of rotifers fed on a standard culture diet (a mixture of live baker's yeast and blue green algae), a commercially available culture diet (Culture Selco), and the algal feed of the current invention is shown in Table 6.

Table 6: Production rate, doubling time, egg production and general appearance of rotifers fed different culture feeds.

Diet	**Production Rate** (rotifers/mL/day^{-1})*	**Doubling Time** (days)	**Egg Production** (high/low**)	**Appearance***
Algal feed	51.4	4.28	High	+/-
Culture Selco	31.5	7	Low	-
Yeast+live algae	96.5	2.27	High	+

* Average of the first 4 days of culture

** High egg production was defined as >70% of the rotifers with eggs.

***General rotifer appearance was determined as follow: (+) sign was given when rotifers appeared swimming fast, carrying more than one egg and water culture was clean; (-) sign was given when rotifers appeared swimming slow, carried one egg or none, and the culture water was murky.

Example 6. Formulation of algal feed with probiotic *Lactobacillus rhamnosus* for rotifer culture

[056] Algal feed was made as described in Example 1, and freeze dried *Lactobacillus rhamnosus* was obtained from Morinaga Milk Industry Co., LTD. (Kanagawa, Japan).

[057] Rotifer feed was formulated by mixing 89.9 g of brewers yeast, 9 g of *Schizochytrium*, 1 g of *Padina*, and 0.1 g of *L. rhamnosus* (Table 7). All ingredients were mixed on a dry weight basis and mill-ground to produce fine particles at sizes between about 5-15μm.

Table 7. Rotifer feed composition (g dry weight/100g)

Brewers yeast	89.9
Schizochytrium sp.	9
Padina sp.	1
L. rhamnosus	0.1

[058] Rotifers were fed with a daily ration of 1 g dry matter/million rotifers. The culture conditions included open air tube aeration, a temperature of 28±1 °C, a salinity of 20 ppt, pH 8±0.5, oxygen saturation 100±5%, and a culture volume of about 20-100 L. Daily diet rations were added 4 –5 times/day.

[059] Temperature (°C), oxygen (% saturation), pH, salinity (ppt), and the number of rotifers/ml were measured daily. The daily rotifer harvest was approximately 30% of the number of rotifers in the culture. Fig. 1 shows the daily production of rotifers fed on a standard culture diet (a mixture of live baker's yeast and blue green algae), a commercially available culture diet (Culture Selco), and the algal feed with probiotic lacto bacteria of the current invention.

Example 7. Formulation of algal feed with brewer's yeast and *Schizochytrium* for rotifer culture

[060] Dried brewers yeast was obtained commercially from Williams Bio-products (Pekin, IL. USA). DHA-rich *Schizochytrium* sp. was obtained from Advanced BioNutrition Corp. (Columbia MD. USA). The rotifers were grown as described in Example 6, except for the specific additions as described in Table 8. The diets containing brewers yeast and *Schizochitrium* at a 90:10 ratio (two different types of brewers yeast) closely matched the control diet, which was supplied with fresh algae and yeast. Additions of olive extract or *Padina* extract did not significantly change the number of rotifers per mL over 3 days inoculation. All of these diets bested the Culture Selco.

Table 8. Comparison of rotifer counts per milliliter after three days of culture

Diet Description	Rotifers/mL
Control diet with algae and yeast	48.0
Brewers yeast 2/*Schizochytrium* at 90:10	40.5
Brewers yeast 1/*Schizochytrium* at 90:10	39.0
Brewers yeast 1/*Schizochytrium* at 90:10 with olive extract	36.9
Brewers yeast 1/*Schizochytrium* at 90:10 with *Padina* extract	33.9
Culture Selco	30.0

References

[061] The specification is most thoroughly understood in light of the following references, all of which are hereby incorporated by reference in their entireties.

U.S. Patent Documents

6,451,567	Barclay, W.	September 17, 2002
6,372,460	Gladue and Behrens	April 16, 2002
6,103,225	Barclay, W.	August 15, 2000
5,739,006	Abe, et al.	April 14, 1998
5,688,500	Barclay, W.	November 18, 1997

5,158,788	Lavens, et al.	October 27, 1992
5,047,250	Prieels, et al.	September 10, 1991

Other References

Abu-Rezq T et al. (2002) Studies on the effect of using the rotifer, Brachionus plicatilis, treated with different nutritional enrichment media and antibiotics on the growth and survival of blue-fin sea bream, Sparidentex hasta (Valenciennes), larvae. Aquaculture Res 33:117-128.

Barclay W (2000) Methods of aquaculture by feeding larval shrimp *Thraustochytrium* and/or *Schizochytrium* microflora. In: U.S. Patent No. 6,103,225; Omegatech, Inc., USA.

Barclay W (2002) Fermentation process for producing long chain omega-3 fatty acids with euryhaline microorganisms. In: U.S. Patent No. 6,451,567 B1. Omegatech, Inc., USA.

Barclay W, Zeller S (1996) Nutritional enhancement of n-3 and n-6 fatty acids in rotifers and Artemia nauplii by feeding spray dried Schizochytrium sp. J. World Aquaculture Soc. 27:314-322.

Bolton ET (1982) Intensive marine bivalve cultivation in a controlled recirculated seawater prototype system. In. University of Delaware Sea Grant Publication, p 165.

Curé K, Gajardo G, Coutteau P (1996) The effect of DHA/EPA ratio in live feed on the fatty acid composition, survival, growth and pigmentation of turbot larvae Scophthalmus maximus L. Improvement of the Commercial Production of Marine Aquaculture Species 26:57-67.

Dabrowski K, Culver D (1991) The physiology of larval fish. Aquaculture Mag 17:49-61.

D'Souza FML, Knuckey RM, Hohmann S, Pendrey RC (2002) Flocculated microalgae concentrates as diets for larvae of the tiger prawn Penaeus monodon Fabricius. Aquaculture Nutrition 8:113-120.

Fukusho K, Hirayama KE (1992) The First Live Feed - Brachionus plicatilis. In. The Oceanic Institute., Honolulu, HI.

Fulks W, Main KL (1992) Rotifer and microalgae culture systems, 364.

Furuita H, Takeuchi T, Uematus K (1998) Effects of eicosapentaenoic and docosahexaenoic acids on growth, survival and brain development of larval Japanese flounder (Paralichthys olivaceus). Aquaculture 161:269-279.

Gladue RM, Behrens PW (2002) DHA-containing nutritional compositions and methods for their production. In: U.S. Patent No. 6,372,460.

Hernandez CM, Portilla Y, Fernandez Palacios H, Gonzalez JA (1986) Maintenance and mass culture of the rotifer Brachionus plicatilis O.F. Mueller, 1786, Bs strain, in the Canary Islands. Inf. Tec. Inst. Esp. Oceanogr.

Hirata H, Yamasaki S, Kawaguchi T, Ogawa M (1983) Continuous culture of the rotifer Brachionus plicatilis fed recycled algal diet. Hydrobiol. 147:269-270.

Hirayama K, Maruyama I (1991) Vitamin B sub(12) content as a limiting factor for mass production of the rotifer Brachionus plicatilis. Larvi' 15:101-103.

Hirayama K, Maruyama I, Maeda T (1989) Nutritional effect of freshwater Chlorella on growth of the rotifer Brachionus plicatilis. Rotifer Symposium:186-187.

Kanazawa A (1992) New directions in microdiet research in Japan. Isr. J. Aquacult. Bamidgeh 44:123.

Kanazawa A (1993) Finfish Hatchery In Asia: Importance of dietary docosahexaenoic acid on growth and survival of fish larvae. In: Proceedings Of Finfish Hatchery In Asia, pp 87-95.

Kitajima C (1983) Actual examples of mass cultures. In: Koseikaku K (ed) The Rotifer Brachionus plicatilis-Biology and Mass Cultue. Japanese Fisheries Society, pp 102-128.

Lavens P, Coutteau P, Sorgeloos P, Vandamme E (1992) Feed for aquaculture. In: USPTO 5,158,788. Synfina-Oleofina, S.A., USA.

Lie O et al. (1997) Nutritional composition of rotifers following a change in diet from yeast and emulsified oil to microalgae. Aquacult. Int. 5:427-438.

Maruyama I, Hirayama K (1993) The culture of the rotifer Brachionus plicatilis with Chlorella vulgaris containing vitamin B sub(12) in its cells. J. World Aquacult. Soc., 24, 194-198. J. World Aquaculture Soc. 24:194-198.

Nagata WD, Whyte JNC (1992) Effects of yeast and algal diets on the growth and biochemical composition of the rotifer Brachionus plicatilis (Mueller) in culture. Aquacult. Fish Manage. 23:13-21.

Place A, R., Harel M (2002a) Use of Arachidonic acid for enhanced culturing of fish larvae and broodstock. In: PCT WO 00219839, UMBI.

Place AR, Harel M (2002b) Use of arachidonic acid for enhanced culturing of fish larvae and broodstock. In: US Pat. Appl. 20020110582 A1.

Planas M, Cunha I (1999) Larviculture of marine fish: problems and perspectives. Aquacult. 177:171-190.

Satuito CG, Hirayama K (1986) Fat-soluble vitamin requirements of the rotifer Brachionus plicatilis. In: The First Asian Fisheries Forum. Proceedings Of The First Asian Fisheries Forum, Manila, Philippines, pp 619-622.

Satuito CG, Hirayama K (1991) Regulation of the amino acid and fatty acid contents of baker's yeast to improve its nutritional value for the population growth of the rotifer Brachionus plicatilis. Bull. Fac. Fish Nagasaki Univ. Chodai Suikenpo:13-20.

Verginelli R, Marin N, Lodeiros C (1994) [Mass culture of the rotifer Brachionus plicatilis under three different microalgae diets.]. Rev. Latinoam. Acuicult. 43:68-71.

Watanabe T (1993) Importance of docosahexaenoic acid in marine larval fish. J. World Aquaculture Soc. 24:152-161.

Watanabe T, Kitajima C, Fujita S (1983a) Nutritional value of live food organisms used in Japan for mass culture of fish: A review. Aquacult. 34:115-143.

Watanabe T, Tamiya T, Oka A, Hirata M, Kitajima C, Fujita S (1983b) Improvement of dietary value of live foods for fish larvae by feeding them on omega 3 highly unsaturated fatty acids and fat-soluble vitamins. Bull. Jap. Soc. Sci. Fish./Nussuishi. 49:471-479.

Yoshimura K, Hgiwara A, Yoshimatsu T, Ktajima C (1996) Culture technology of marine rotifers and theimplications for intensive culture of marine fish in Japan. Mar. Freshwater Res. 47: 217-222.

Yoshimura K, Kitajima C, Miyamoto Y, Kishimoto G (1994) Factors inhibiting growth of the rotifer Brachionus plicatilis in high density cultivation by feeding condensed Chlorella. Nippon Suisan Gakkaishi/Bull. Jap. Soc. Sci. Fish. 60:207-213.

I Claim:

1. An aquaculture feed comprising yeast and microalgae, or components thereof.
2. The aquaculture feed of claim 1, wherein the mean particle size ranges from about 5 μm to about 100 μm.
3. The aquaculture feed of claim 1, wherein the feed components are dry mixed and ground to a fine powder.
4. The aquaculture feed of claim 1, wherein the yeast comprises from about 30 to about 95 percent of the feed.
5. The aquaculture feed of any of claims 2-4, wherein the yeast are chosen from *Saccharomyces* spp., *Saccharomyces cerevisiae*, *Phaffia* spp., *Phaffia rhodozyma*, *Pichia* spp., *Pichia pastoris*, *Kluyveromyces* spp., *Kluyveromyces aestuarii*, *Kluyveromyces marxianus*, and *Kluyveromyces yarrowii*.
6. The aquaculture feed of any of claims 2-4, wherein the yeast is brewer's yeast.
7. The aquaculture feed of claim 1, comprising at least about 5 percent by weight from microalgal sources.
8. The aquaculture feed of claim 7, wherein the microalgal source is chosen from *Tetraselmis* sp., *Tetraselmis suecica*, *Myrmecia* sp., *Myrmecia bissecta*, *Lyngbya* sp., *Lyngbya majuscula*, *Cytospora* sp., *Scenedesmus* sp., *Scenedesmus obliquus*, *Scytonema* sp., *Scytonema hofmanni*, *Nostoc* sp., *Nostoc weissfloggia*, *Chaetoceros* sp., *Chaetoceros lauderi*, *Ecklonia* sp., *Ecklonia maxima*, *Dunaliella* sp., *Dunaliella salina*, *Dunaliella tertiolecta*, *Dunaliella bardiwal*, *Pseudoanabaena* sp., *Anabaena* sp., *Prorocentrum* sp., *Prorocentrum minimum*, *Polysiphonia* sp., *Polysiphonia denudata*, *Spirulina* sp., *Arthrospira* sp., *Spirulina platensis*, *Aphanotheae* sp., *Aphanotheae nidulans*, *Hydrodictyon* sp., *Hydrodictyon reticulatum*, *Navicula* sp., *Navicula delongei*, *Phaeodactylum* sp., *Phaeodactylum tricornutum*, *Pseudonitzschia* sp., *Nitzschia* sp., *Nitzschia navis-varingica*, *Chlorella* sp., *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Chlamydomonas* sp., *Chlamydomonas reinhardii*, *Prennioporia* sp., *Prennioporia medullaeparis*, *Pandorina* sp., *Pandorina morum*, *Isochrysis* spp., *Isochrysis galbana*, *Schizochytrium* sp., *Crypthecodinium* sp., *Crypthecodinium cohnii*, and *Thraustochytrium* sp.

9. The aquaculture feed of claim 7, wherein the microalgae are selected from *Schizochytrium* sp., *Crypthecodinium* sp., *Crypthecodinium cohnii*, *Thraustochytrium* sp., and components thereof.
10. The aquaculture feed of claim 1, further comprising a probiotic element.
11. The aquaculture feed of claim 10, wherein the probiotic element is chosen from *Lactobacillus* sp., *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Pseudoalteromonas* sp., and *Pseudoalteromonas undina*.
12. An aquaculture feed comprising yeast, microalgae, and macroalgae.
13. The aquaculture feed of claim 12, wherein the mean particle size ranges from about 5 μm to about 100 μm.
14. The aquaculture feed of claim 12, wherein the feed components are dry mixed and ground to a fine powder.
15. The aquaculture feed of claim 12, wherein the yeast comprises from about 30 to about 95 percent of the feed.
16. The aquaculture feed of any of claims 12-15, wherein the yeast is chosen from *Saccharomyces* spp., *Saccharomyces cerevisiae*, *Phaffia* spp., *Phaffia rhodozyma*, *Pichia* spp., *Pichia pastoris*, *Kluyveromyces* spp., *Kluyveromyces aestuarii*, *Kluyveromyces marxianus*, and *Kluyveromyces yarrowii*.
17. The aquaculture feed of any of claims 12-15, wherein the yeast is partially digested.
18. The aquaculture feed of claim 12, comprising at least about 5 percent by weight from macroalgae .
19. The aquaculture feed of claim 18, wherein the macroalgal source is chosen from *Tetraselmis* sp., *Tetraselmis suecica*, *Myrmecia* sp., *Myrmecia bissecta*, *Lyngbya* sp., *Lyngbya majuscula*, *Cytospora* sp., *Scenedesmus* sp., *Scenedesmus obliquus*, *Scytonema* sp., *Scytonema hofmanni*, *Nostoc* sp., *Nostoc weissfloggia*, *Chaetoceros* sp., *Chaetoceros lauderi*, *Ecklonia* sp., *Ecklonia maxima*, *Dunaliella* sp., *Dunaliella salina*, *Dunaliella tertiolecta*, *Dunaliella bardiwal*, *Pseudoanabaena* sp., *Anabaena* sp., *Prorocentrum* sp., *Prorocentrum minimum*, *Polysiphonia* sp., *Polysiphonia denudata*, *Spirulina* sp., *Arthrospira* sp., *Spirulina platensis*, *Aphanotheae* sp., *Aphanotheae nidulans*, *Hydrodictyon* sp.,

Hydrodictyon reticulatum, Navicula sp., *Navicula delongei, Phaeodactylum* sp., *Phaeodactylum tricornutum, Pseudonitzschia* sp., *Nitzschia* sp., *Nitzschia navis-varingica, Chlorella* sp., *Chlorella pyrenoidosa, Chlorella vulgaris, Chlamydomonas* sp., *Chlamydomonas reinhardii, Prennioporia* sp., *Prennioporia medullaeparis, Pandorina* sp., *Pandorina morum, Isochrysis* spp., *Isochrysis galbana, Schizochytrium* sp., *Crypthecodinium* sp., *Crypthecodinium cohnii*, and *Thraustochytrium* sp.

20. The aquaculture feed of claim 12, comprising at least about 0.5 percent by weight from macroalgae .
21. The aquaculture feed of claim 20, wherein the macroalgal source is chosen from *Laminaria* spp., *Padina* sp., *Gracillaria* sp., and *Ulva* sp.
22. The aquaculture feed of claim 20, wherein the macroalgal source comprises a macroalgal extract.
23. The aquaculture feed of claim 22, wherein the macroalgal extract comprises an ethanolic extraction product.
24. The aquaculture feed of claim 12, further comprising a probiotic element.
25. The feed of claim 24, wherein the probiotic element is chosen from *Lactobacillus* sp., *Lactobacillus casei, Lactobacillus rhamnosus, Pseudoalteromonas* sp., and *Pseudoalteromonas undina.*
26. The aquaculture feed of claim 12, wherein the yeast is partially digested.
27. A method comprising feeding a cultured species with a feed as in claim 1.
28. A method comprising feeding a zooplankter species with a feed as in claim 1.
29. The method of claim 28, wherein the zooplankter species is chosen from brine shrimp, rotifers, *Artemia* spp., *Artemia salina, Artemia franciscana, Brachionus* spp., *Brachionus plicatilis*, copepods, and cladocerans.

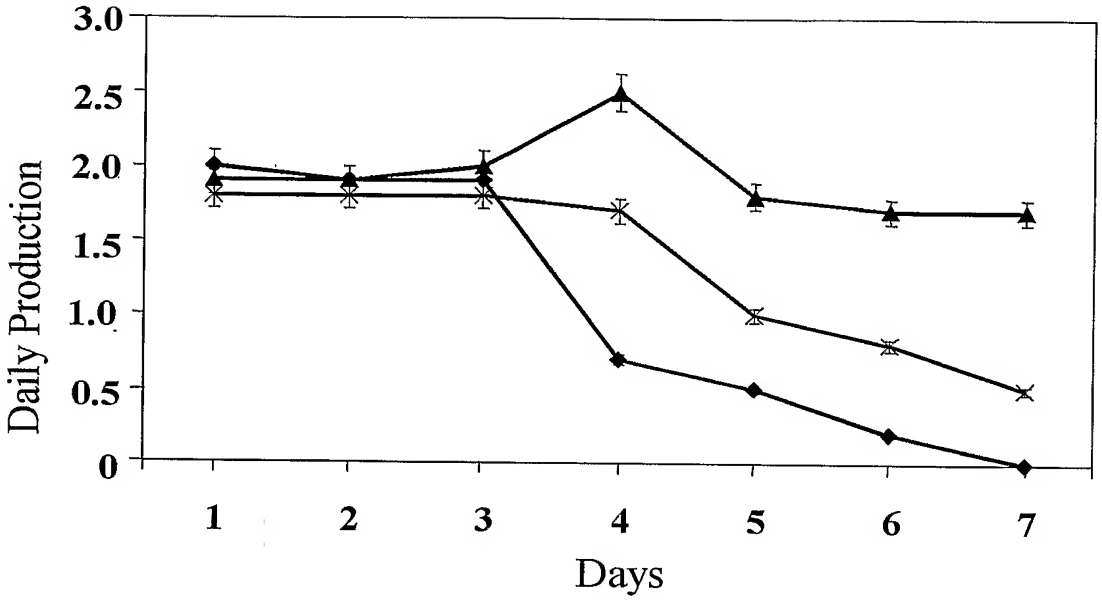

Fig. 1